INTRODUCTION TO NGS TECHNOLOGIES

DNA SEQ , RNA-SEQ, CHIP-SEQ, HI-C, METAGENOMICS, SINGLE CELL SEQ

What is Next-generation sequencing (NGS)?

Next-generation sequencing (NGS) is a massively parallel sequencing technology that offers ultra-high throughput, scalability, and speed. The technology is used to determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA.

SOME NGS TECHNIQUE:

- ILLUMINA SEQUENCING
- PYRO-SEQUENCING
- ROCHE 454 SEQUENCING
- ION TORRENT SEQUENCING

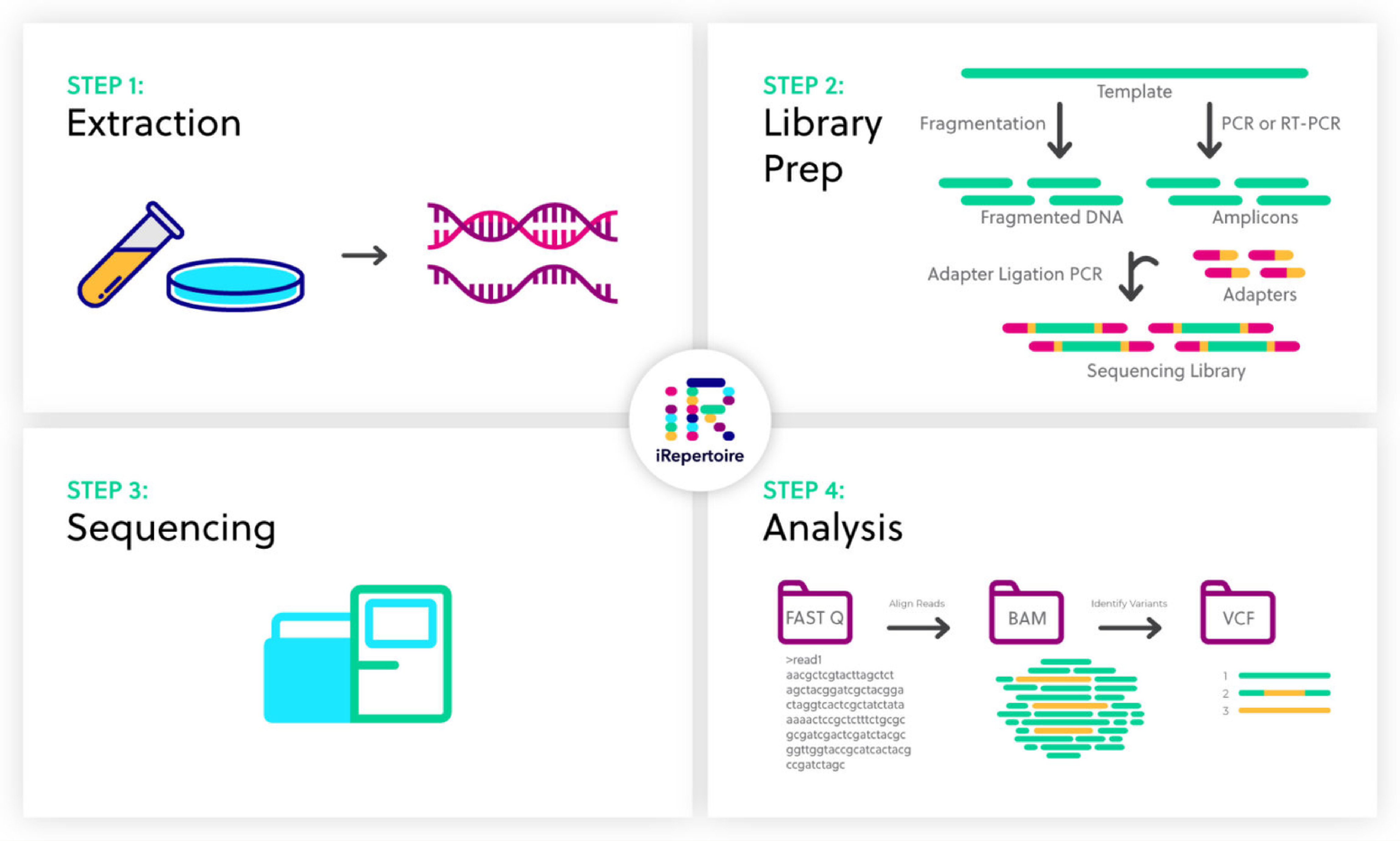
PERSONALISED MEDICINE

CLINICAL DIAGNOSTICS

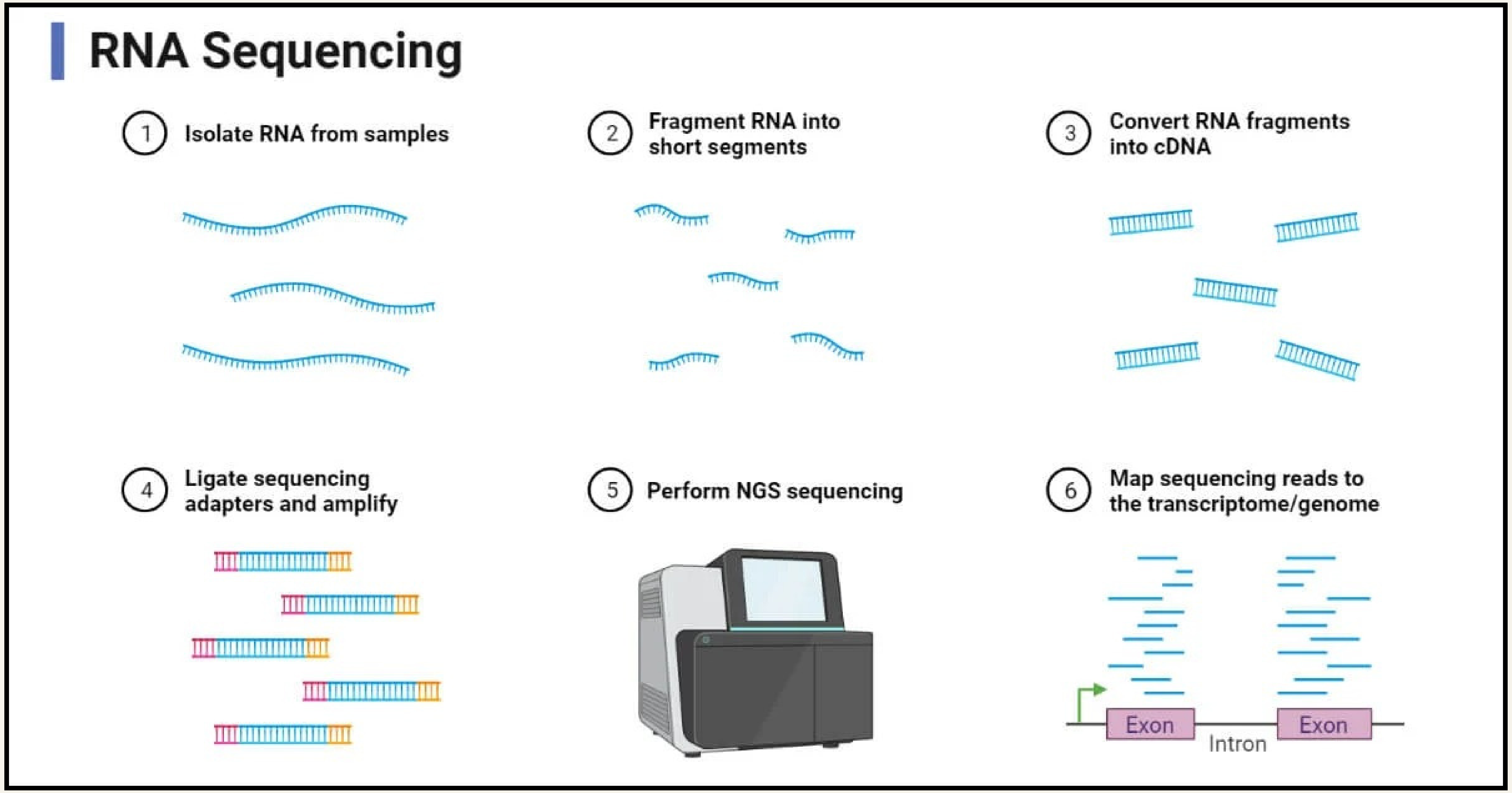
GENETIC DISEASES

Key Applications of NGS

1. DNA Sequencing (DNA-seq)



2. RNA Sequencing (RNA-seq)



3. Chromatin Immunoprecipitation Sequencing (ChIP-seq)

- *Definition*: ChIP-Seq is a technique to study protein-DNA interactions in the genome.
- *Significance*: Helps understand gene regulation and protein binding in chromatin.
- *Key Point*: Identifies where proteins bind to DNA, aiding in gene expression studies.

ChIP-Seq Workflow

Fixation

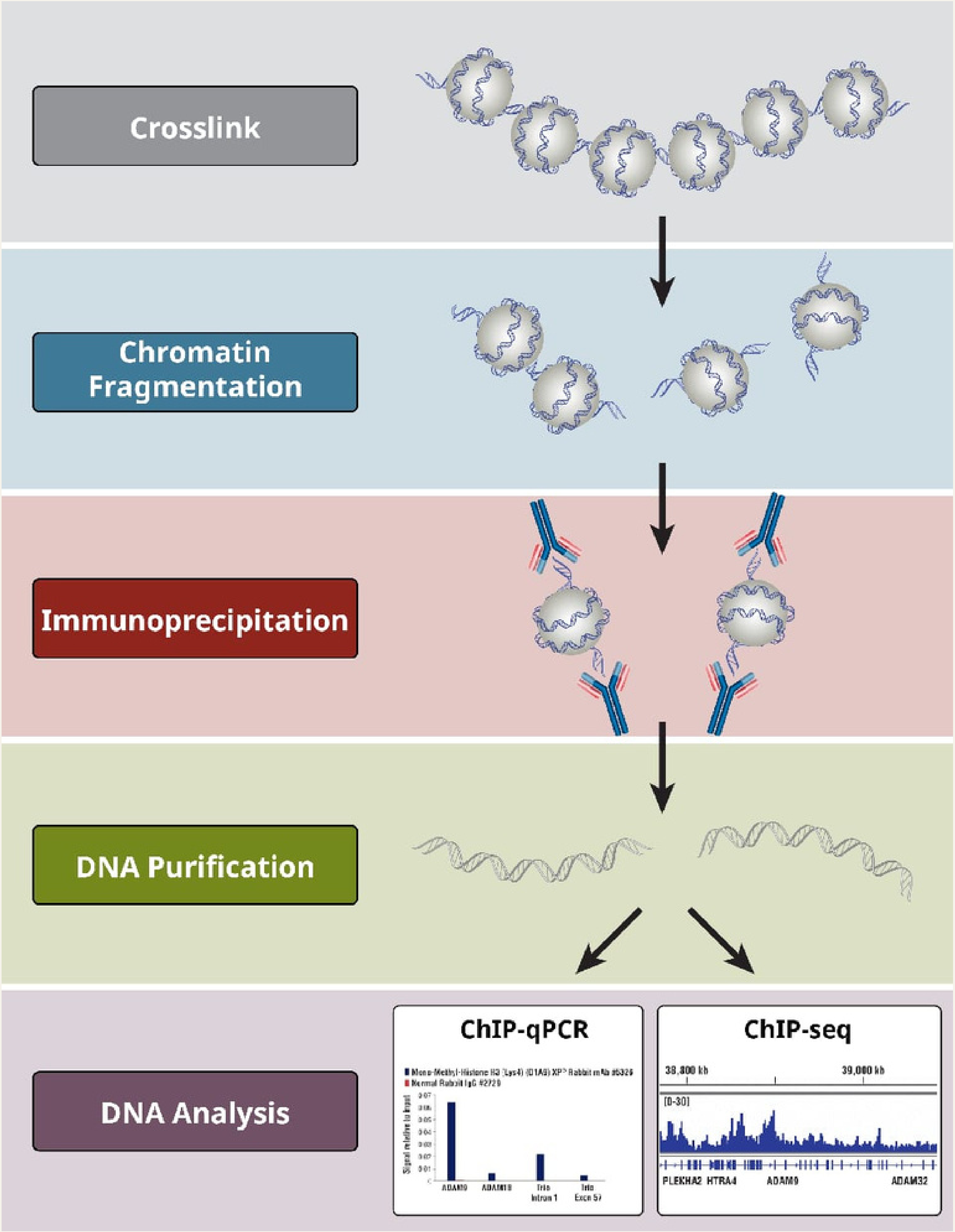
DNA Fragmentation

Immunoprecipitation

Library Construction

Sequencing

Data Analysis



4. High-throughput Chromosome Conformation Capture Sequencing (Hi-C Seq)

- *Definition*: Hi-C Seq reveals 3D genome organization and DNA interactions.
- *Significance*: Provides insights into spatial relationships between DNA segments.
- *Key Point*: Helps understand how genes are folded and interact within the cell nucleus.

Hi-C Seq

Chromatin Cross-Linking

↓

DNA Fragmentation

↓

End Repair and Biotin Labeling

↓

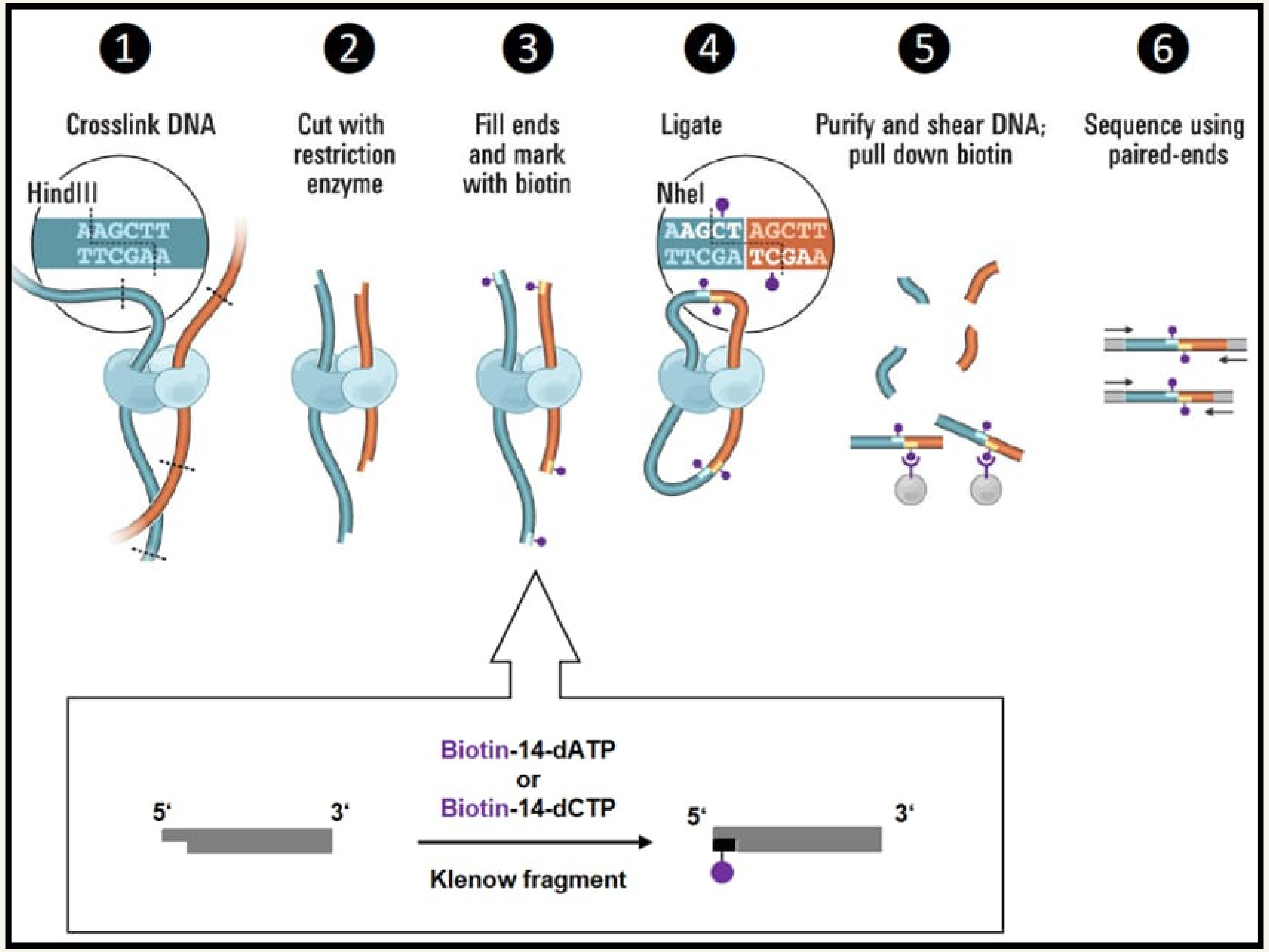
DNA Ligation

↓

Sequencing

↓

Data Analysis



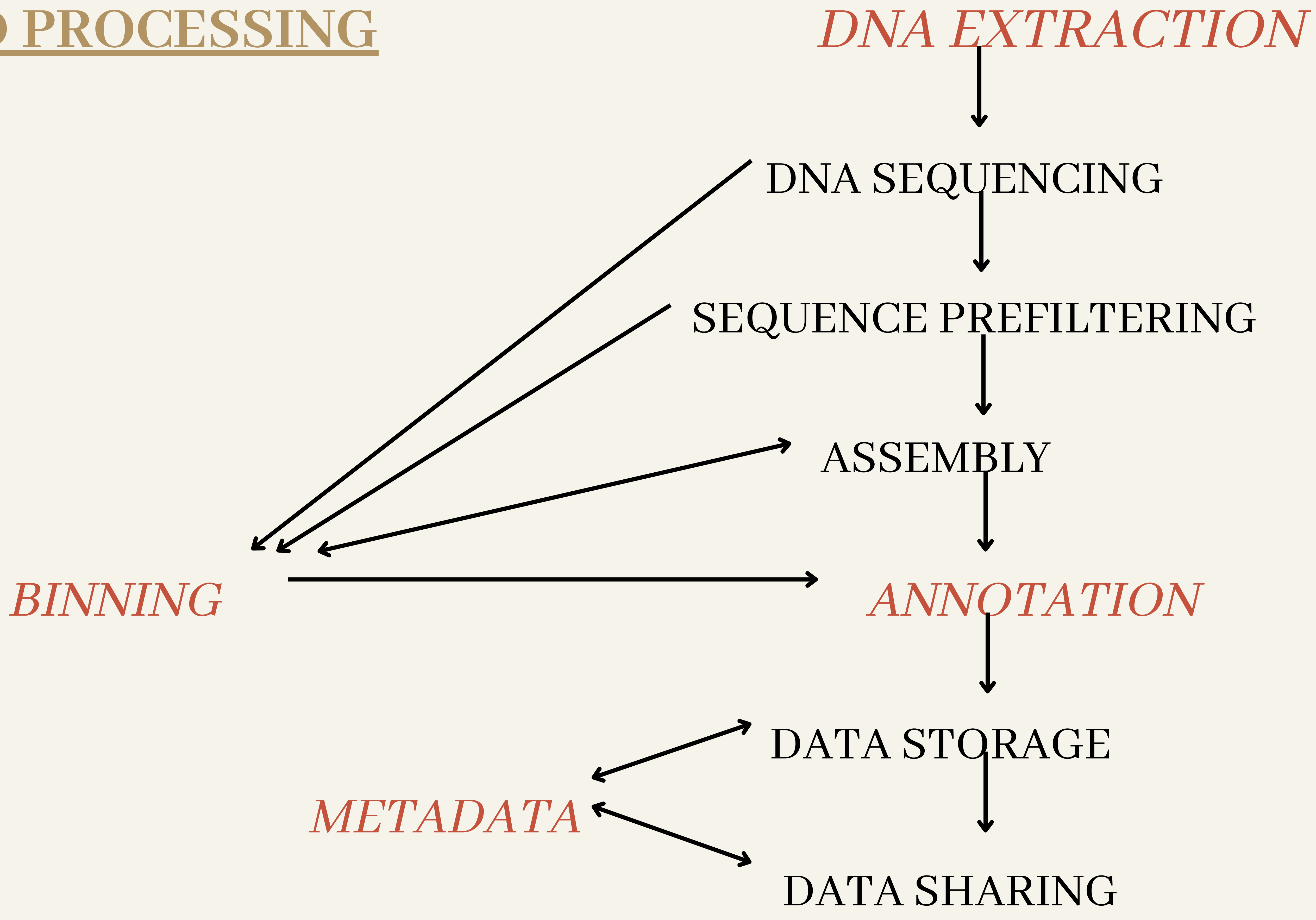
Metagenomics

Metagenomics is defined as the direct genetic analysis of genomes contained within environmental samples, enabling the study of microbial communities without the need for cultivation.

Metagenomics is crucial for studying microbial communities in various samples like soil, water, air, and animal gut microbiota.

Metagenomics provides a comprehensive understanding of microbial diversity and function while identifying new microorganisms and genes.

SAMPLING AND PROCESSING



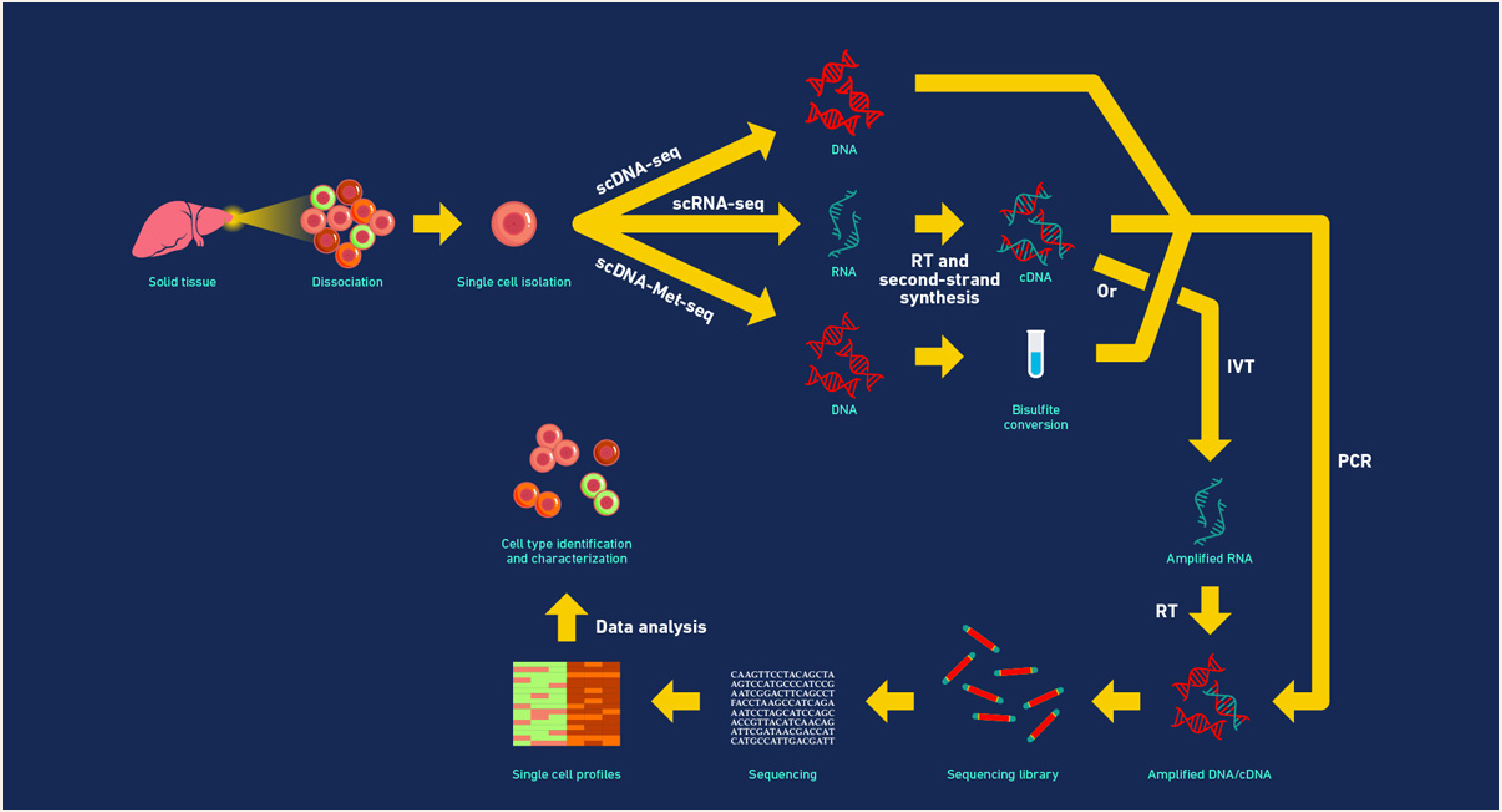
FLOW DIAGRAM OF METAGENOMIC SEQUENCING

SINGLE CELL SEQUENCING

Single-cell sequencing is a cutting-edge genomic technique that allows the analysis of the genomes or transcriptomes of individual cells, providing a high-resolution view of cell-to-cell variation.

It overcomes the limitations of traditional population-level measurements, allowing for in depth analysis of individual cells and their functions.

Single-cell sequencing has revolutionized the field by providing insights into gene expression, cellular heterogeneity, and complex cellular systems at an unprecedented level of detail.



SINGLE CELL SEQUENCING WORKFLOW

FOUR MAIN STEPS OF SINGLE CELL SEQUENCING TECHNOLOGY

1.Isolation of single cells from a cell population.

2) Extraction, processing and amplification of the genetic material of each isolated cell.

3) Preparation of a “sequencing library” including the genetic material of an isolated cell.

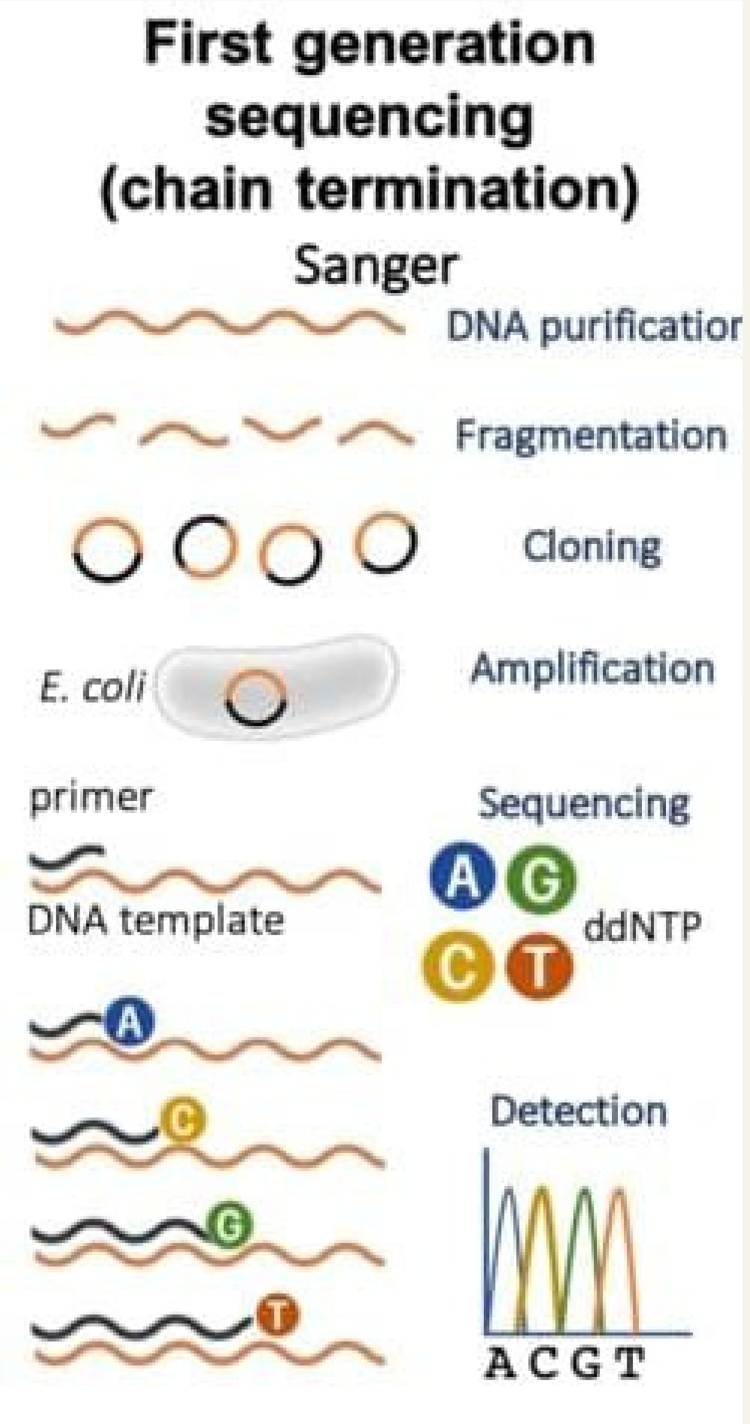
4) Sequencing of the library using a next-generation sequencer.

APPLICATIONS:

- Cancer Research
- Immunology
- Drug discovery and development
- Stem cell Research

Comparison of output, accuracy and types of errors of first, second and third generation sequencing technologies.

FIRST GENERATION SEQUENCING



OUTPUT

- The sequencing of clonal DNA populations
- Low Output
- Short read sequencing

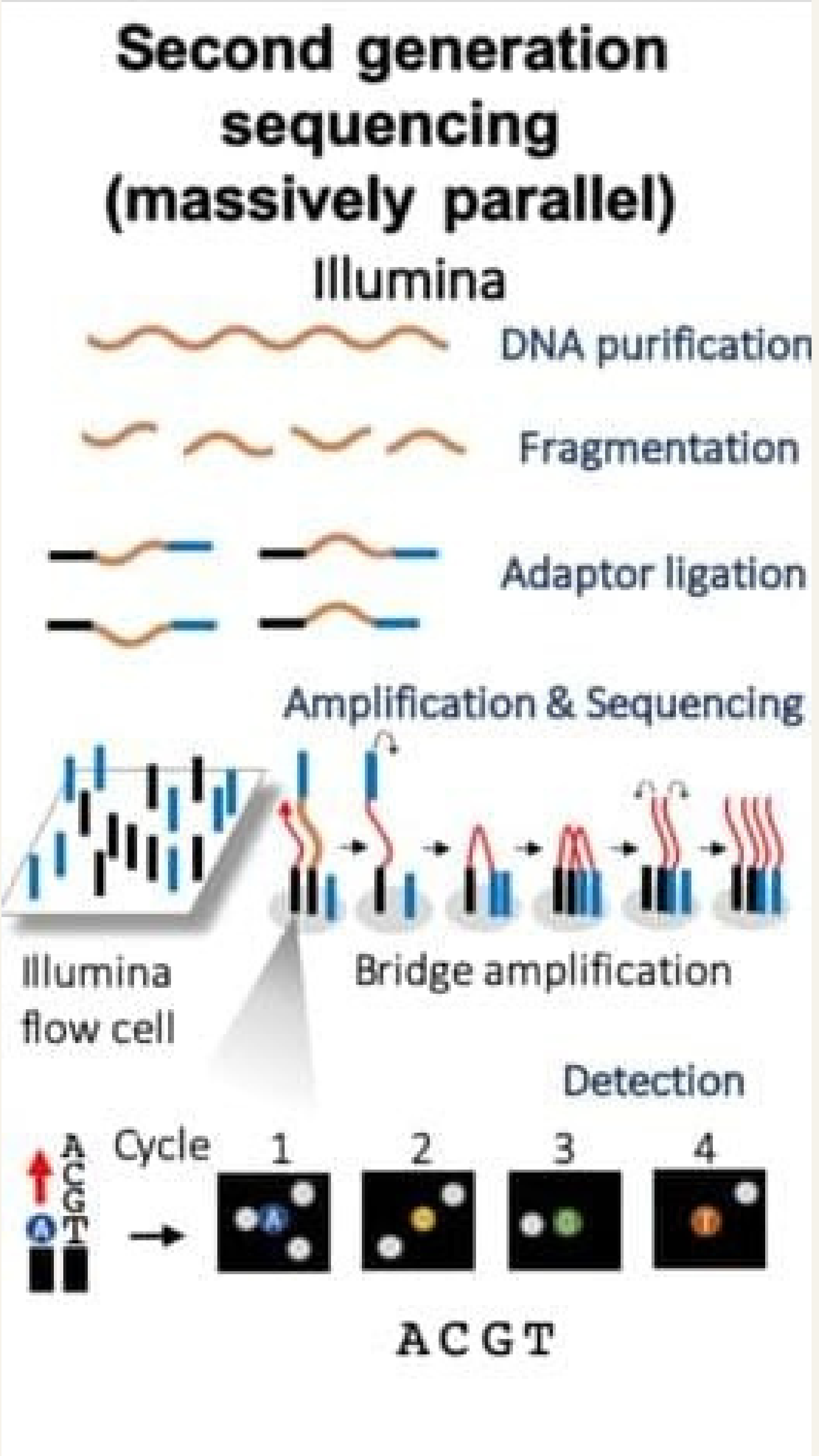
ACCURACY

- Accuracy but limited scalability and high cost
- Accuracy >99%

ERROR

- Error rate of 0.001%

SECOND GENERATION SEQUENCING



OUTPUT

- Reads around 36–600 base pairs long
- High Output
- Short read sequencing

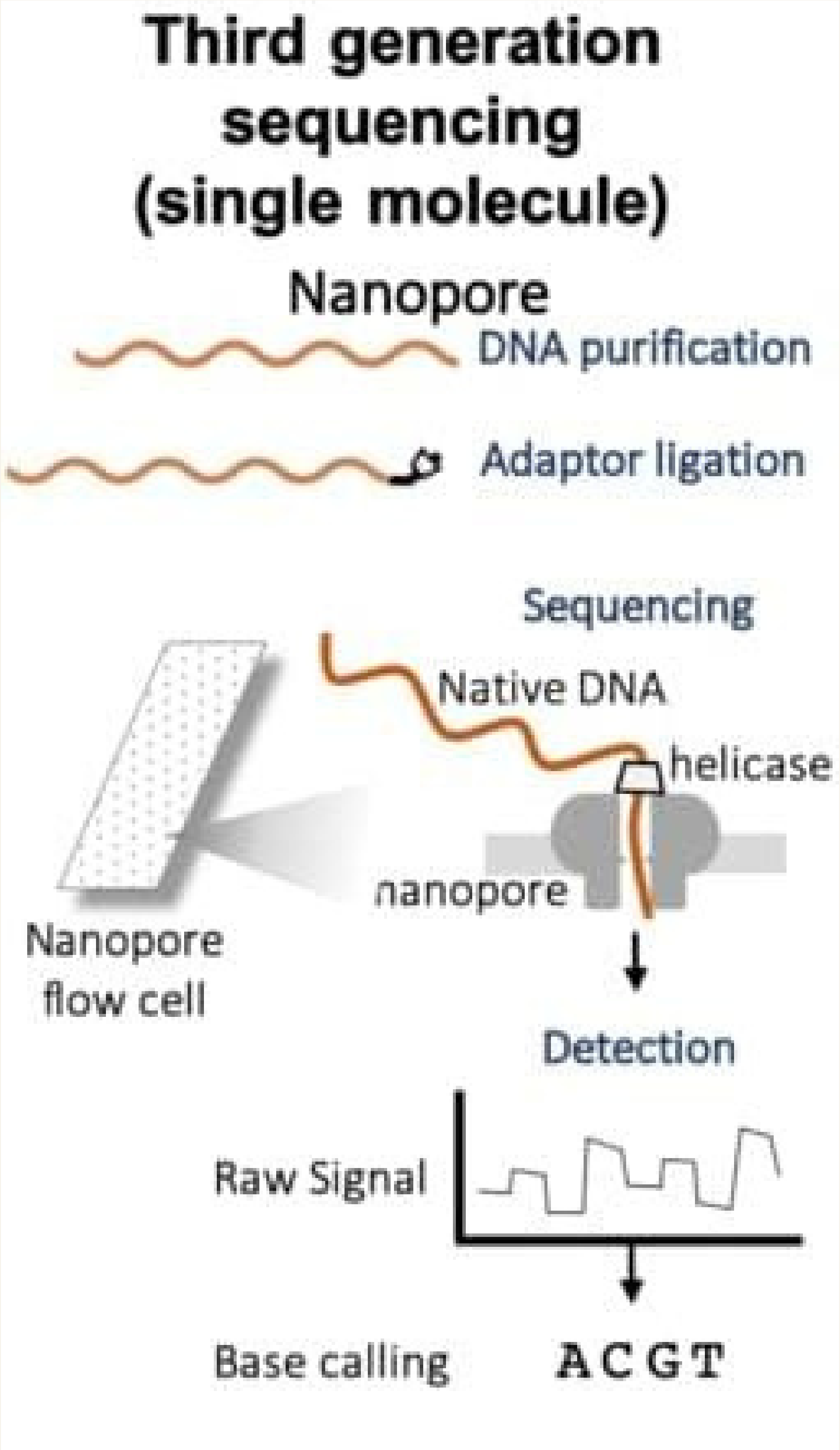
ACCURACY

- High accuracy levels
- Accuracy >99%

ERROR

- Error rate of around 1%

THIRD GENERATION SEQUENCING



OUTPUT

- Longer reads at lower costs and in shorter times
- High Output
- Long read sequencing

ACCURACY

- Accuracy 90-95%

ERROR

- Highter error rate compared to the other types